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Simulation and evaluation of different statistical functions for describing lag time distributions of a bacterial growth curve☆

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ABSTRACT

The objective of this study was to simulate the lag time distribution of individual bacterial cells in a population incubated under an isothermal condition. Spores of *Clostridium sporogenes* PA 3679 inoculated to ground beef were incubated at 37 °C to develop a growth curve and determine its apparent lag time. The lag times of individual cells were simulated using normal, lognormal, Gumbel, gamma, Weibull, and exponential distributions. The growth process was modeled using the McKellar model. A 4th order Runge–Kutta method was used to solve the McKellar model in conjunction with the Nelder–Mead method. The objective of the numerical analysis was to search for the parameters in each lag time distribution model and the kinetic parameters (specific growth rate and mean lag time) of the growth model such that a minimized residual sum of squared errors (RSS) was found.

Although different in shapes, the simulation results showed that the performance of normal, lognormal, Gumbel, and gamma distributions was practically the same when used to fit the growth curve, yielding the same estimates of initial and stationary phase bacterial concentrations, specific growth rate, and mean lag time as well as RSS. The lognormal, Gumbel, and gamma distributions of the lag times were slightly skewed to the right of the mean. The standard deviations of the lag times with these distributions were relatively small, suggesting that cells may leave the lag phase almost simultaneously. The Weibull and exponential distributions were also suitable for describing the distribution of lag times, but with larger standard deviations. In general, all these statistical distributions were found suitable for simulating the distribution of lag times of individual cells in a population.

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1. Introduction

The growth of microorganisms in food usually exhibits three distinctive phases, including the lag, exponential, and stationary phases. When a bacterial culture is inoculated or exposed to a new environment, the bacteria may experience a lag phase, in which the cells gradually adjust before they begin to divide actively. Within the lag phase, the bacterial counts usually do not exhibit a noticeable change. The duration of a lag phase is determined by the type, strain, temperature, and prior history as well as the substrate in which bacteria grow. For spore-forming microorganisms, the lag phase may include germination and outgrowth of spores before the first vegetative cell begins to grow and divide.

Once a growth curve is developed, the population's apparent lag time can be easily determined from the curve (Buchanan et al., 1997). However, biological variation may exist among the individual cells of the bacterial population (Buchanan et al., 1997), allowing individual cells to possess different abilities to emerge from the lag phase. This is particularly true for spores. Many hypotheses have been proposed to describe the formation and duration of the bacterial lag phase in a growth curve (Baranyi, 1998, 2002, 2009; Baty and Delignette-Muller, 2004; Buchanan et al., 1997; Swinnen et al., 2004; Guillier and Augustin, 2006; Koutsoumanis, 2008; Olofsson and Ma, 2011). One of the hypotheses is the individual cell lag time theory (Baranyi, 1998, 2002; Guillier et al., 2005; Kutalik et al., 2005; Pin and Baranyi, 2006). Based on this theory, the formation of lag phase in a bacterial culture is determined by each cell and each cell may leave its lag state individually. Each cell would need to accumulate critical substance before it can grow and start dividing. Once a cell leaves its lag phase, it enters the exponential phase, starting to grow and divide immediately.

The objective of this work was to evaluate the applicability of and compare different statistical distributions for describing the distribution of individual lag times in a population of bacterial cells under

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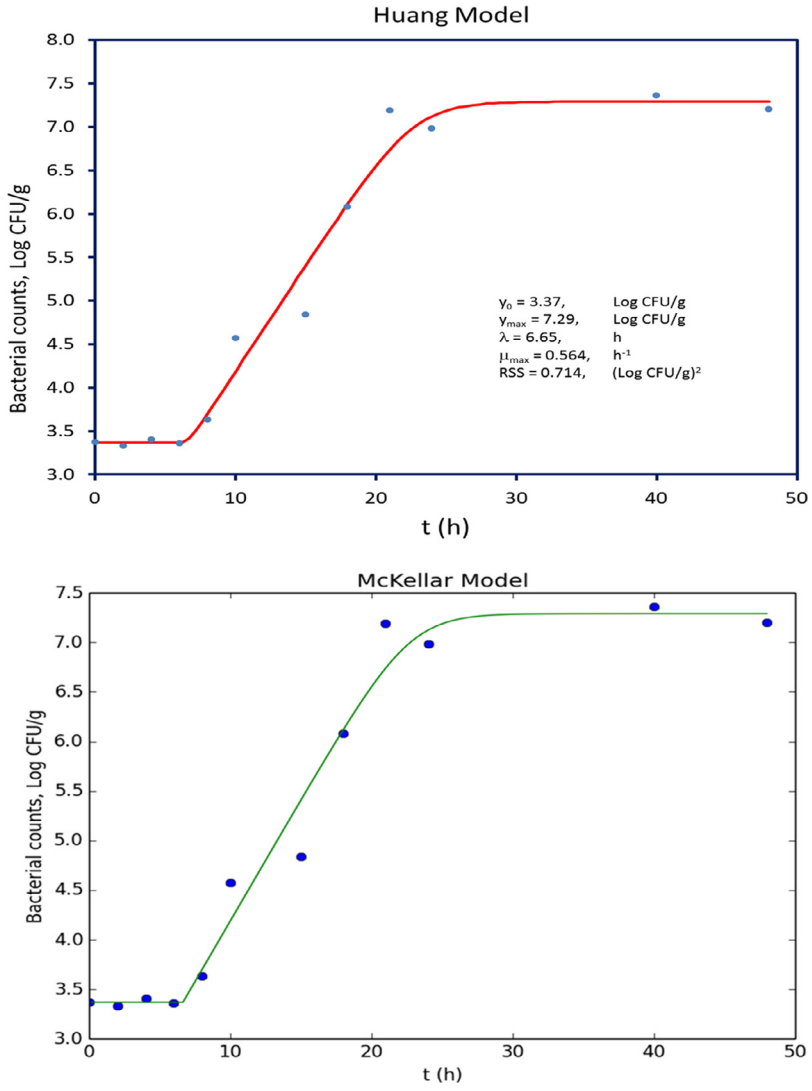

Fig. 1. Results of curve-fitting using the Huang model and McKellar model.

a constant temperature condition. Individual lag times were simulated with different statistical distributions, and numerical optimization was used to determine the parameters in each distribution and the kinetic parameters for the growth curve. The results of this study may help to develop more accurate predictive models and methods to estimate and predict the growth of microorganisms in foods.

2. Materials and methods

2.1. Hypothesis

It is assumed that a pure culture of a certain bacterium is inoculated to a substrate that contains sufficient nutrients to support its growth. The bacterial culture is incubated under a constant temperature condition that permits bacterial growth. Under a suitable condition, the bacterial growth gradually progresses from the lag phase, through the exponential phase, and then reaches the stationary phase.

If all cells are in the lag phase at time zero and leave the lag phase at the same time, the growth of bacteria can be described by a two-compartment model (Eqs. (1) and (2), McKellar, 1997). In Eq. (1), t is the incubation time (h); C_0 is the initial bacterial count (CFU/g); C_{max} is the bacterial count at the stationary phase (CFU/g); λ is the lag phase; and μ_{max} is the specific growth rate (h^{-1}). This model suggests that the cells start exponential growth immediately after exiting the lag phase when $t > \lambda$. This model implies that all cells leave the lag phase simultaneously.

$$C = C_0, \quad \text{for} \quad t \le \lambda \tag{1}$$

$$\frac{dC}{dt} = \mu_{max} C\left(1 - \frac{C}{C_{max}}\right), \quad \text{for} \quad t > \lambda \tag{2}$$

According to the framework proposed by Buchanan et al. (1997), the lag phase of a cell can experience two distinct periods. In the first period (t_a), the cell senses the need for physiological adjustments and adapts to the new environment. In the second period (t_m, or generation time), the cell goes through a series of metabolic reactions to generate sufficient energy and needed biological components to replicate (the first generation). The time that a cell spends in a lag phase is the sum of t_a and t_m. In this study, the lag time is denoted as λ, which is $t_a + t_m$.

If it is assumed that each individual cell of the bacterial culture exhibits different lag times, the lag time is unique for each cell. During incubation, the cells emerge from the dormant state in the order of lag times, with the cells possessing shorter lag times emerging from the dormant state sooner than those of longer lag times. Assuming that all cells are in the dormant state immediately after inoculation,

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